
Senescence Arrest of Endopolyploid Cells Renders Senescence into One Mechanism for Positive Tumorigenesis

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Abstract

The present chapter analyses the cellular genomic status and type of cell divisions of pre-senescent cell populations shortly before the senescent phase. The purpose was to identify cellular mechanisms contributing to genomic unstable escape cells from senescence, in likeness with cancer recurrence from dormancy/remission periods. Primary diploid, human fibroblast cells were passage-extended to dysfunctional telomere-induced senescence (TAS) during which endopolyploidization occurred shortly before senescence. These cells either reduced genomic content to unstable near-diploid cells by irregular whole complement co-segregation, perpendicularly to the cytoskeleton axis or by nuclear fragmentation into multinuclear cells. The endo-division offspring-cells acquired genomic/chromosomal instability (CIN) from inheritance of endo-division traits, and gained proliferative freedom from contact inhibition by the perpendicularly of the endo-division. Other cells during this low proliferative period showed chromosomal aberrations and mitotic restitution, before a general change to senescent cells. The senescent phase showed change to typical cytoplasmic rich, amorphous flat cells, which were beta-galactosidase positive. Several types of escape mechanisms were observed (most frequent: nuclear bud-offs) which were associated with mitotic activity, albeit with limited propagation. These cells

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showed slight cell-shape changes from cell polarity change—a trait caused by endo-division perpendicularity. In experiments designed to simulate tumor therapy-induced genomic damage (to kill cells) endopolyploid cycling activity was increased before change to large amorphous, senescent cells. Young senescent escape-cells used the bud-off escape route, whereas escapes from old cells showed triangular cells in close contact with the senescent cells, which grew into three dimensional (3-D) tumor-like spheres. These observations were discussed as to tumor recurrence after prolonged dormancy/remission periods.

Keywords

Escape cells • 3-D growth • Kalikreins • Contact inhibition • Cell polarity • Skewed cytoskeleton • Meiotic-like

Introduction

Senescence is a physiological and morphological change of cells to a none-replicative state and occurs for both in vitro and in vivo cells. The cells gain large amounts of cytoplasm and change into amorphous flat cells with a nuclear to cytoplasmic ratio forbidding to mitotic activity. Normal proliferating cells in renewal tissues (skin/gut) reach a phase in their replicative ability that is hampered by dysfunctional telomeres (ends of chromosomes) that can no-longer support mitotic fidelity and the cells cease proliferation with change to the senescent cell morphology. This process was first shown for in vitro extended passages of normal diploid cell strains (Hayflick and Moorehead 1961). During the cell expansion-period telomeres progressively become short from loss of their specific repetitive DNA sequences until critically short, which the cell perceives as genomic damage and signals arrest by a senescence response (telomere associated senescence – TAS) (Walen 2007a; Davoli et al. 2010). Lately the senescence status with its secretory phenotype and complex physiology has been linked to several aging dis-

eases, among them, tumorigenesis (Collado et al. 2007; Vergel et al. 2011).

Beta-galactosidase (beta-gal) activity in lysosomal vehicles accompanies the morphological changes to large flat cells, which with staining at pH 6.0 has become a widely used marker of senescent cells, which can also be visualized as small, clear bubbles in phase contrast microscopy of unstained cells (Walen 2011) Other markers have been proposed from nucleolar changes. This long know condition of cytoplasmic digestive vehicles associated with the transition from replicative to non-replicative senescence cells (Hayflick and Moorehead 1961) was recently suggested to involve autophagy (Young et al. 2009). This digestive process apparently breaks down old pro-proliferation cytoplasmic-compounds with “recycling” synthesis to an anti-proliferative condition of senescent cells Other genomic and epigenomic changes involve activation of genes for p53, p21, p16ARF or Rb, p21, p16INK4a, and development of heterochromatic foci (SAHF) with effect of gene-silencing (Collado et al. 2007; Collado and Serrano 2010; Vergel et al. 2011). Many experiments, designed to show reversion of the senescent state, have in general been negative, and when pre-neoplastic cells were shown to enter the senescent state, the conceptual leap to senescence as an anti-tumor, irreversible mechanism became generally accepted (Beausejour et al. 2003; Collado et al. 2005).

Crucial to the present title are a recent review on induced therapeutic genomic damage and its association to endopolyploidy and “leaky” senescence (Mosieniak and Sikora 2010). It cites numerous references many of which supporting present observations/conjectures and will be used as an overall reference in this chapter. Additionally, two works from in vivo/in vitro studies also showed: (1) a link between drug-therapy induced genomic damage (to kill cells) and change to polyploidy with senescence entry, which generally is referred to as premature/accelerated senescence (ACS), and (2) that preserved biopsy materials of neoplasia were positive for the senescent cell phenotype and beta-gal (Roberson et al. 2005; te Poele et al. 2002). Roberson et al. (2005) further showed that the

“reversion” from senescence to genetically changed cells was a very rare (10^{-6}) event, called “escape”. The escape clones showed correlated up-regulation of cyclin B1 and Cdk1 (cyclin and kinase) which are indicators of mitotic activity, but routes of escape from senescence were not identified.

Mechanistic Escape Routes from Telomere Associated Senescence

We studied young (1–3 weeks) senescent cells in vitro by extended passages to the senescent phase of two primary, human diploid fibroblast cell strains (Walen 2004, 2005, 2006). Stained and phase contrast photography showed that nuclei can move in the large, flat cells to the cell membrane/envelope and there become wrapped in membrane for exocytosis (i.e., nuclear bud-off). This process most often led to long, thin extensions of cytoplasm away from the mother cell with mitosis often occurring at the tip (Fig. 18.1a–c). When the nuclear tip region was separated from the mother cells, the resulting cells, became flattened from surface attachment and showed one centrosome and a normal mitotic sequence, albeit to very limited population doublings (Walen 2005). Other escape cells returned to the flat cell morphology, indicating senescent-cell genomic heterogeneity. Recently another escape route from thinly seeded lysosomal positive senescent cells gave details of cytoplasmic-reduction aided by lysosomes, not known from earlier works (Fig. 18.2a–j). The difference was due to fixation in absolute methyl alcohol instead of in Carnoy’s solution. These cells showed cytoplasmic discard as large, blebs on the surface of mitotic cells (metaphase and anaphase) involving large and small lysosomal vacuoles. The alignment of the lysosomal bodies at the base of the blebs indicates a controlled and selective autophagic process. Figure 18.2e shows a large disconnected bleb which apparently separated from the mother-cell without injury to the cell envelope. The blebs are likely formed by cell envelope puffing-extensions, enclosing various amounts of cytoplasm which was occurring in cells with endopolyploid mitotic

activity, suggesting discard of anti-proliferative chemicals (Young et al. 2009). This picture is in contrast to similar non-mitotic flat cells, negative for lysosomal presence undergoing necrosis with bits and pieces of digested cell membrane surrounding the cells (Fig. 18.2h–j).

Other cells, multinucleated cells (MNCs) from fragmentation of giant cells ($>8n$), in these senescent cell populations, also showed lysosomal “alignment” associated with discard of non-viable necrotic products (Fig. 18.2j), and MNCs furrowing into individual cells, and triangularly shaped cells appeared to tolerate significant amounts of lysosomes (Fig. 18.2k, l). These latter cells different from the striated fibroblasts suggests endopolyploid origin (see below). Other MNCs in pre-senescence showed the nuclear bud-off process which could be increased by a trypsin-digestive reduction of the extra cellular matrix (Walen 2002, 2008). In tumorigenesis, giant cells and MNCs are often present with the extreme example of osteoclast-like cells. Information about radiation-induced MNCs and their potential contribution to tumor genetic variability from their rather unique segregation system is forthcoming (Erenpreisa et al. 2011). Additionally it is known that tumorigenic cells secrete kallikreins (metalloproteinases) that can act like trypsin for potentiating a bud-off process. Various kallikreins have become markers of tumorigenic cells with the best known test being PSA for prostate specific antigen. Nevertheless, all in vitro observations mentioned here, together demonstrate that the nuclear bud-off mechanism is a general phenomenon for attainment of a nuclear to cytoplasmic ratio compatible with mitotic activity. Alternatively, cycling cells stopped in their course (by senescence) are poised for continuation of cycling. This cellular quest to stay cycling active (cells can survive in G0) applies to tumor recurrence and to mitotic slippage of G2 cells into S for endoreplication to endopolyploidy. However, escape cells from senescence must synthesize their own genomic-controlled cytoplasm for mitotic entry, which for MNCs in tumor-cell lines was shown to involve significant increase in

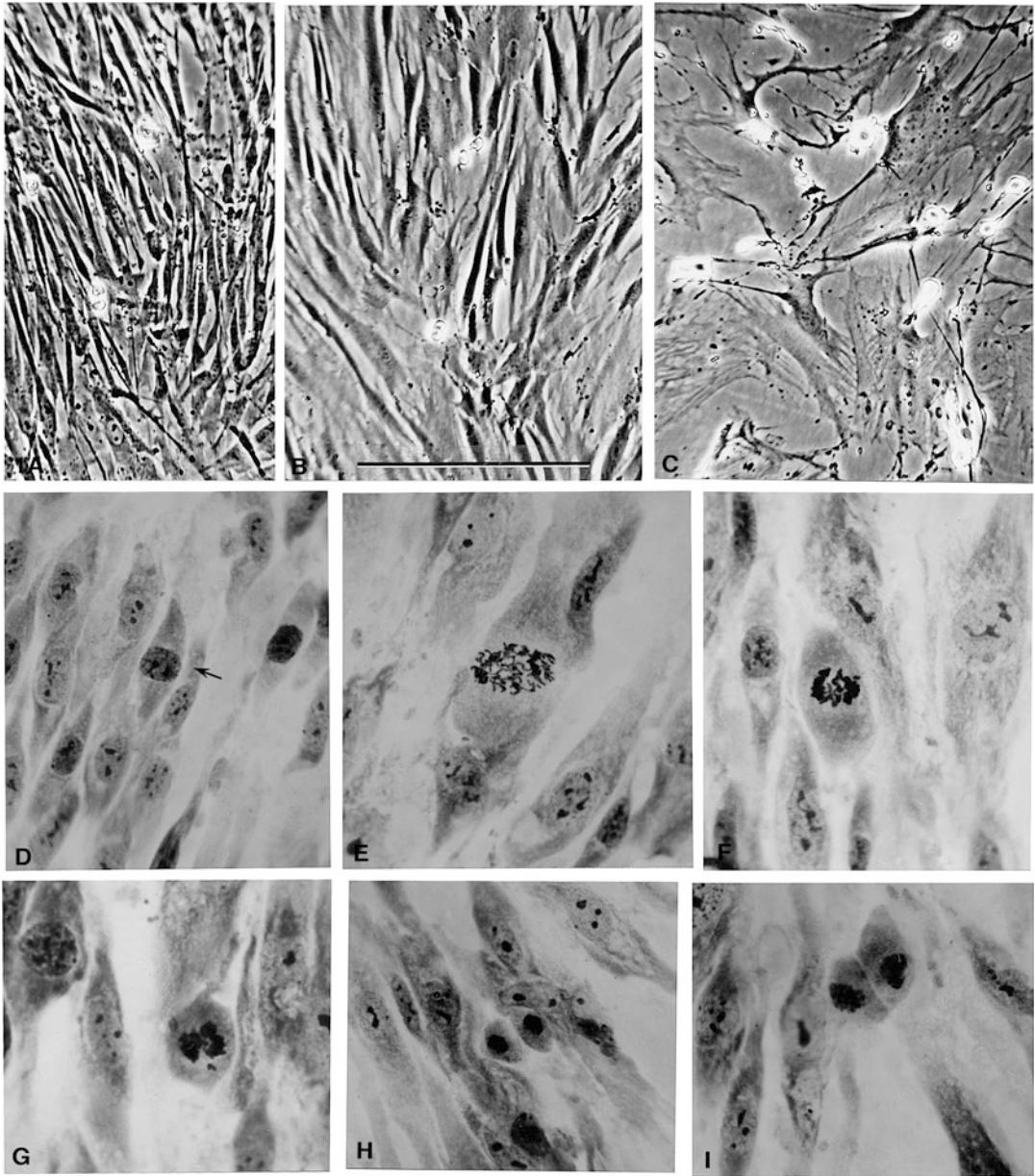


Fig. 18.1 Normal fibroblast (a) change to beginning senescence flat cell morphology (b) and escape cells with mitotic activity from nuclear bud-offs from senescent cells (c). Perpendicular nuclear and endo-divisions relative to the cytoskeleton axis: (d) nuclear orientation, (e, f)

endotetraploid prophase and beginning anaphase, (g, h, i) endotetraploid first division products in perpendicular orientation to the surrounding cells. Magnifications: a–c=620 \times ; d–i=990 \times

nucleolar ribosomal activity (Erenpreisa et al. 2011). Roberson et al. (2005) showed for lung cancer cells that escape cells were molecularly more similar to the senescence arrested cells than cells of origin.

The next two sections present conflicting observations and suggestions of the cellular status of senescence, and of how endopolyploid cells with irregular, non-mitotic cell division contributes to genomic heterogeneity in senescent cell populations.

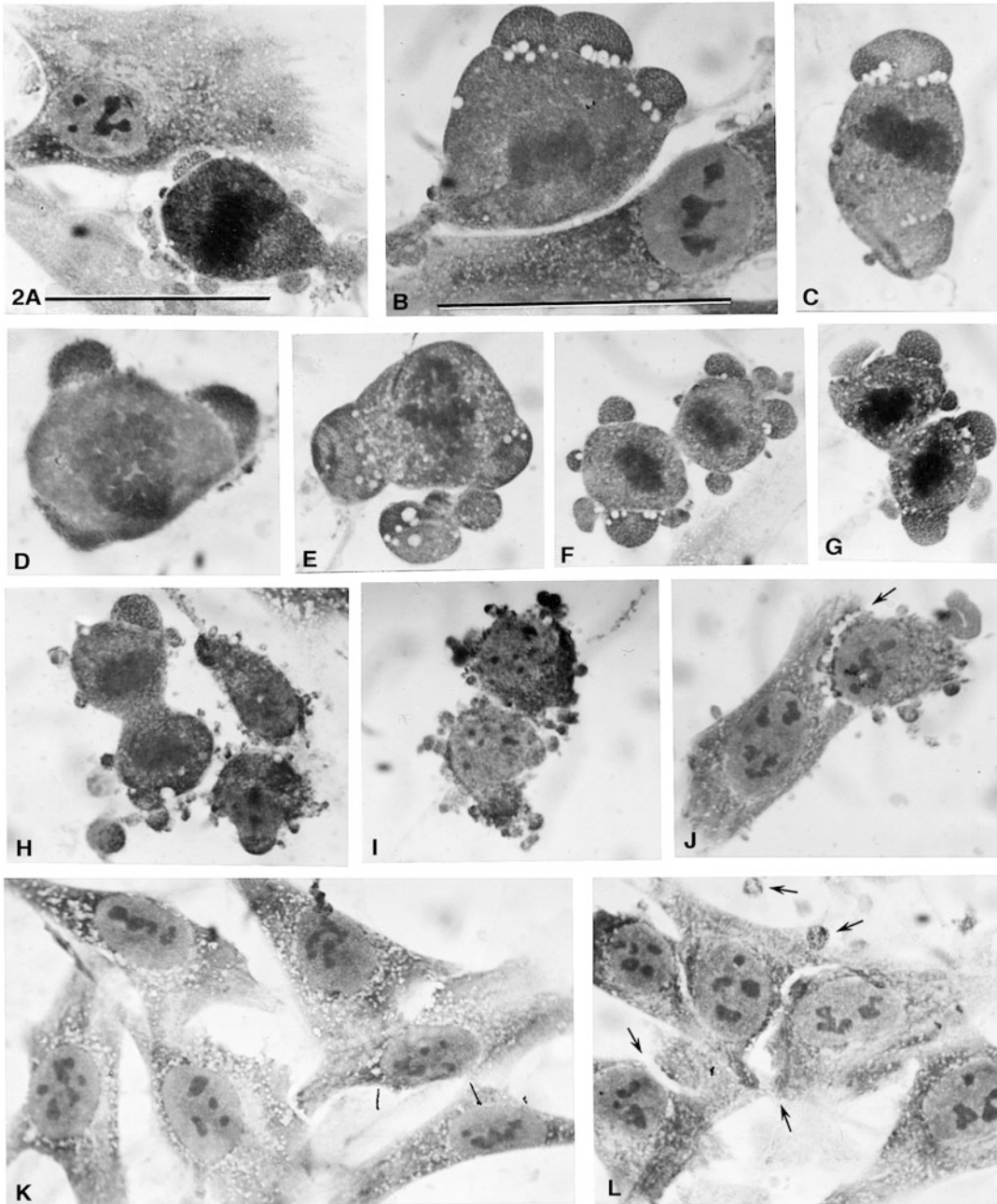


Fig. 18.2 Cytoplasmic reduction by membrane-bound blebs discarded from isolated senescence cells with lysosomal presence. (a) A large senescence interphase cell close to a mitotic cell with blebs, (b, c) mitotic cells with rows of large lysosomes at the base of the blebs, (d, e, f, g) mitotic cells in meta- ana- telo- phase to interphase with blebs, of note: the anaphase figure (e) is oriented perpen-

dicularly to the cytoskeleton axis, (h, i) necrosis in the absence of lysosomes, (j) lysosomal "aided" discard of a necrotic product from an MNC, (k) triangular cells with abundant content of micro-lysosomes without apparent deterioration, (l) cytoplasmic furrows between nuclei of multinucleated cells (MNCs), arrows point to discarded blebs. Magnifications: a, c-l=2,475 \times and b=3,217 \times

Escape from Senescent Cells Preceded by Cell Growth from Increased Endopolyploidy

A rather confusing issue is whether therapy-induced tumor-cell senescence can be compared to replicative senescence (TAS) (Shay and Roninson 2004). There is a tendency to equate beta-gal positivity with senescence induction when in fact such happenings are known from simple cell culturing to be a cell-arrest response. Cytoplasmic granulation, as an indicator of culture decline (Hayflick and Moorehead 1961) was in earlier works during cell culture media-developments ascribed to lysosomal activity. Personal experience with proliferative well-beings of primary cell cultures has always involved a check for presence of granulation, since proliferation-arrest from toxic media components had been encountered. But, with a quick medium replacement the cells regained cytoplasmic-clarity and proliferative power, which demonstrate that lysosomal presence is an indicator of cell-well-being, and not an exclusive marker of senescence (Walen, unpub.). Shay and Roninson (2004) discussed the genetic and epigenetic differences between TAS and STASIS (stress-associated induced senescence) with STASIS being a TAS-like response from “exogenously induced rapid G1 growth arrest”. Vergel et al. (2011) stressed the molecular differences in effectors of the senescence program (e.g., p53 and p16ARF is mostly compromised in cancers). Therefore, in the following presentation to avoid confusion, the terms dormancy/remission will be used for other types of senescence.

There are also other great conflicts about the genotypic and phenotypic status and metabolic activities of cells that are in replicative senescence (TAS): (1) Entry of only diploid cells (2n/2C/G1/G0) versus such cells together with an amount of cells arrested in G2 (either diploid 2n/4C or tetraploid 4n/4C – no cytometry difference). Nuclear size differences support a mixture of G1/G0 and G2 cells (Walen 2006; Matsumura et al. 1979). (2) Questionable DNA synthesis in senescent cells by observation of different cell

types labeled with tritiated thymidine (TH3) (Matsumura et al. 1979; Beausejour et al. 2003). (3) Possible physiologic and genomic differences between young (weeks 1–2) and old (5–8 weeks) senescent cells are a neglected subject. This latter possibility was demonstrated by young TAS cells showing DNA repair (γ H2AX) and heterochromatic (chromocenters) foci that disappeared in old senescent cells (deep senescence) (Chen and Ozanne 2006). Exposure of the old cells to H2O2 (induced DNA damage) resulted in development of new repair foci together with 53BP1 protein which showed p53 activation. Repaired telomeres were suggested, with likely association with the slow uptake of TH3 as a DNA repair synthesis (Matsumura et al. 1979). If true, aged senescent cells would have greater probability of showing re-growth from escape cells than young cells. The cell-escape from the dormant lung tumor cells mentioned above was a late (3–4 weeks) cell-escape (Roberson et al. 2005), which is also a trend for tumor recurrence from remission/dormancy.

These latter facts prompted an experimental design for senescence induction that would simulate the genome-wide damage often associated with therapy agents and thus, not only be restricted to telomere-associated damage (TAS-treated) (Walen 2011). This induction was achieved by exposure of near-senescent primary human cells to culture medium deficient in glutamine. Earlier studies of amino acid deficiency had shown chromosomal aberrations from breakage and reunions, and high frequencies of endo-tetra-octopolyploidy and giant cells (Freed and Schatz 1969). The shortly (2–3 days) treated cells were propagated (two to three passages) in recovery medium (glutamine positive) to senescence, which again was rather sudden with change to large, amorphous flat cells, containing high levels of lysosomal vehicles. These TAS-treated cells, on their way to senescence showed increased endo-divisions as compared to TAS alone, and expressed a tendency for cells to be on top of each other (Fig. 18.3f), and also showed small dysplastic foci of cells with cell-shape changes from cell polarity change. The senescent cultures were not sub-cultured, only

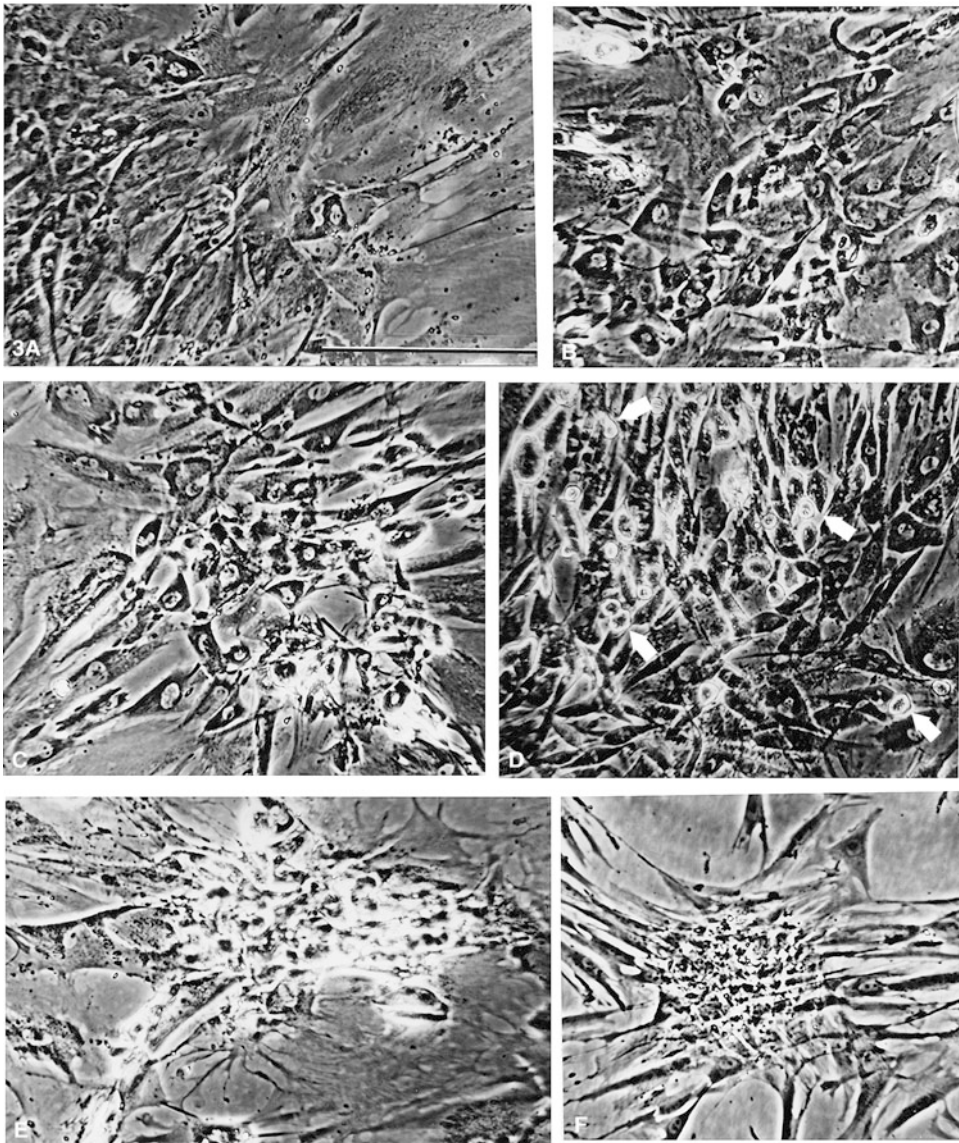


Fig. 18.3 Escape cell proliferation of cell polarity changed cells associated with three dimensional (3-D) tumor-like growths. (a–e) Different growth foci in close association with the large amorphous old, flat cells, all showing similar cell-shape-changes and a small con-

densed nucleus, (d) contains many mitoses (*arrows*) and (e) shows beginning to a 3-D structure morphologically very different from an early 3-D senescence-escape growth (f), seemingly with largely retained fibroblastic phenotype. Magnification: All illustrations 2,475×

exposed to frequent media changes and pH adjustments in order to counteract the cell-destructive effects from beta-gal-lysosomes, which was discarded into the medium (Walen 2011).

Young senescent cells demonstrated the nuclear bud-off process, showed gamma-H2AX repair- and senescent associated heterochromatic foci (SAHF – gene silencing) (Mosieniak and Sikora 2010). But with increasing maintenance-time to

more than a month, the two types of foci had gradually disappeared and amounts of lysosomes were significantly reduced. For a fraction of cells, lysosomes were concentrated in a band around the nucleus/nuclei (MNCs), also shown by others as a distinguishing feature of senescent cells. In these TAS-treated 5–8 week old cultures, several different sized clumps of cells appeared floating in the medium, and also surface-attached with three dimensional (3-D) spherical tumor-like growths (Walen 2011). The proliferating cells showed a triangular shape and small condensed nuclei, very different from cells of origin, strongly striated/fibroblastic (Fig. 18.1a versus Fig. 18.3a–d). The surface attached 3-D growths (Fig. 18.3e) appeared to be situated on top of the senescent cells, implicating a cell-central nuclear bud-off mechanism (Walen 2002). These late occurring 3-D growths had a possibility of origin from two types of cells, either with or without added induced genomic damage to that from dysfunctional telomeres. Both conditions however, implicated biological events of unknown nature in the protracted senescence phase. Interestingly, the gamma-H2AX- and SAH-Foci also disappeared with extended dormancy period for preneoplastic adenomas from which escape cells grew into full blown adenocarcinoma cancers (Collado et al. 2005). Both types of escape cells (in vitro/in vivo) had gained different genetic characteristics, and most importantly, regain of proliferative capacity by telomere “repair DNA synthesis” (Chen and Ozanne 2006) and/or by telomerase activation.

Escape Cells Can Harbor Genomic Status for Positive Tumorigenesis

The above statement is based on 3-D growths in liquid media as opposed to colony growth in semi-solid medium commonly used as a cell-transformation assay. These growths with cell-shape changes from change/loss of cell polarity can be a consequence from the irregular (non-mitotic) endopolyploid division-system. There are many studies that link tumorigenesis to poly-

ploidy, but few discriminate between regular polyploidy and endo-polyploidy. These two types have entirely different division systems, – regular with mitosis, and endo-polyploidy – with a two-step meiotic-like division. Moreover, endo-division chromosomes have four chromatids as compared to metaphase chromosomes with two chromatids (Levan and Hauschka 1953; D’Amato 1989; Walen 2006, 2007a, b; Davoli et al. 2010). The 4-chromatid condition was a consequence of re-replication of G2 chromosomes (bichromatid chromosomes) having escaped arrest, caused by genomic damage from short telomeres in pre-senescence (Walen 2007b; Davoli et al. 2010). The first division of the meiotic-like segregation separated the 4-chromatid complexes into two groups of bichromatid chromosomes (e.g., $4n/8C$ into two $4n/4C$ products). This was accomplished by co-segregating whole complements in a perpendicular orientation relative to the cell-polarity axis, the cytoskeleton. Unlike mitosis, this division was independent of the spindle apparatus which without an intervening S-period further divided the 2-chromatid chromosomal groups into four single chromatid diploid-sized nuclei (two $4n/4C$ divisions to four $2n/2C$ nuclei) (Walen 2007b). The endo-division is thus a genome reductional division by reverting back to near-diploid cells.

Before becoming senescent cells the reverted offspring cells with mild cell polarity changes divided with modified mitosis and cell cycles due to genomic instability caused by inherited endo-division traits, merged with the innate mitotic machinery (Walen 2012). Senescence with entry of endopolyploid cells, and endo-division products, together with diploid cells, aberrant or not, is a genetically heterogeneous phase. Escape cells will have probability of giving rise to for example, cancer-cell like proliferation (3-D growth) as observed in the old in vitro senescent cell cultures. Data from in vitro/in vivo treatment of cells/tumors with chemo-agents are increasingly showing or suggesting, (endo)-polyploid senescent cells with reversion to lesser ploidy cells as escape cells in tumor relapse (Puig et al. 2008; Mosieniak and Sikora 2010). Thus, the conjecture that senescence can be a positive contributor

to oncogenesis is based on endopoly-ploidy as a genomically unstable cell status in senescence, and on escape-cells showing 3-D tumor-like growths composed of genetically changed cells with cell polarity change/loss.

Cell Polarity, a Control System Against Unscheduled Cell Growth

Over a century ago progressive cell-shape changes from cell polarity loss in anaplasia during cancer progression all the way to round cells was interpreted to lead to “greater capacity for independent living” (Bignold et al. 2007). Numerous investigations have shown that cancer progression, especially the transition to metastatic cells, is associated with loss of adhesion between cells. In cancer-cell pathology dedifferentiation-associated cell-shape changes plays a major role in classifications of mild, moderate or severe dysplasia. Mechanisms for these early cellular indications of potential cancer-development have not been forthcoming. In normal cell-growth, cell-to-cell adhesion acts as a cell-proliferative control, as, for example, expressed by contact inhibition of fibroblast cells. On the molecular level the normal adhesion (e.g., tight junction) is regulated by diverse adhesion proteins of which E-cadherin is a major inter-cellular molecule. In cancerous growths the level of this protein goes from mildly reduced to complete loss which is correlated with increasing pathological grade to metastatic cancer cells, often expressing round cell-shapes.

In normal skin development the nucleus orients from a parallel basement membrane position to a vertical orientation with associated reorientation of the cytoskeleton proteins (actin, fibrils etc.). This latter re-orientation does not happen in endo-division-associated perpendicularity relative to the cytoskeleton axis (Walen 2011). The first meiotic-like division-products become perpendicularly oriented relative to surrounding cells (Fig. 18.1d-i; out of sequence from space limitation). Notably, the co-segregating genomes move from each other in a parallel direction in agreement with the basolateral cell cortex. The

endo-cell becomes stretched in the mid-zone with gradual flattening of the apical and basal regions. The net effect is collapse/destruction of the cytoskeleton which does not lead to cell arrest or apoptosis, but to continuation of the second meiotic-like division, and to mitosis of the genome reduced, cell-shape changed offspring cells. These observations offer a physical mechanism for disruption of cell-to-cell contact. For example, assuming an E-cadherin “gluing” system in contact-inhibited fibroblasts in pre-senescence, this protein has an external-cell part between cells (the glue) and another short part that goes through the cell envelope and into the cytoplasm where it is tied to the cytoskeleton by bridging catenin-molecules. The endo-division system with genomic movements perpendicularly to the cytoskeleton axis which flattens the mother cell will have destructive effect on the E-cadherin-catenin complex and on the glue between cells. The adenomatous polyposis coli (APC) gene in vivo when mutated (lost) produce tetraploidy-associated with catenin dysfunction, suggesting endotetraploid-divisions to unstable diploid cells in colon cancer (Ceol et al. 2007). Furthermore, foci of dysplastic cells (loss of normal cell orientation) associated with endopolyploid cell cycling in pre-senescence (Walen 2011) suggest a similar route from endopolyploidy for early clinical dysplastic lesions (e.g., dysplastic nevi contain endoreplicated nuclei). Such lesions can increase in severity (pathological grade) by repeated endo-divisions.

Discussion

It must be emphasized that this summary exclusively deals with experimental results from normal, diploid, primary cell strains (L645 & WI-38) for the purpose of identifying *normal* cell changes relevant to a tumorigenic potential. Use of cancer-cell lines are first of all informative to therapy-related issues, but has also been crucial in providing of cancer-related hallmarks (e.g., genomic instability and aneuploidy) – genomic and epigenomic changes which the normal cell must acquire.

The present evaluation of proliferative cells in pre-senescence over three to four passages (with dilution factor 1:2) before senescence-entry clearly demonstrated that the change of diploid cells to endopolyploid cycling cells showed versatility for the creation of some salient tumorigenic features, namely genomic instability and proliferative ability of senescence escape cells (free from contact inhibition). Six main features set endopolyploidy apart from other suggested tumorigenic mechanisms, as most likely to function in cancer initiation and progression: (1) it occurs as a genome damage response in normal aging cells from dysfunctional telomeres, when cancer is at the highest incidence (Collado et al. 2007); (2) the two-step meiotic-like endoreduction division is independent of the spindle apparatus which nullifies arrest from checkpoint controls; (3) the endo-division perpendicularity disrupts cell-to-cell contact from cytoskeleton defects and releases offspring cells from contact inhibited growth; (4) low level endopolyploidy (4n & 8n) revert back to mitotic near-diploid and -tetra-ploid cells by genome reductional division; (5) genome reduced endo-offspring cells acquire CIN from mergence of inherited endo-division traits with the mitotic machinery, which leads to modified mitosis and cell cycles; and (6) endopolyploid cycling is a mitotic-meiosis for primitive organisms (e.g., *Aulachantha*; Walen 2011) and is an inherent evolutionarily preserved property of mammalian cells (e.g., tropho-blastic tissue). Numerous cellular mechanisms have been proposed for the creation of genetic variability, all extracted from their occurrence in cancer cells, with breakage-fusion-bridge cycle (B-F-B) and multiple centrosomes for chromosomal instability and multipolar mitosis being the most frequent suggestions. None of these mechanisms have however, been shown to result in cells with acquired inherent, genomic instability (#5 above), a most crucial characteristic of cancer cells (Mosieniak and Sikora 2010).

The pivotal feature of endopolyploid cell cycling as a mechanism in tumorigenesis is the change to 4-chromatid chromosomes which “demands” orderly segregation (mitotic-meiosis #6 above) – a division system that does not

involve regular mitosis. Currently there is only one study showing a link between endopolyploidization and change to 4-chromatid chromosomes (Davoli et al. 2010) in addition to some pioneering works (Levan and Hauschka 1953; Walen 1965; D’Amato 1989) and our own recent studies. Of note, is that similar to the present normal-cell endopolyploidization process, a “G₂^{endo}”-cell sequence to endopolyploidy from re-replication of therapy-related genomic damage was shown for human tumor cells (Smith et al. 2007). The further step to observations of 4-chromatid chromosomes was not mentioned, and consequently endopolyploid divisions were treated with regular mitosis – interpretations which unfortunately are a general occurrence today (Ceol et al. 2007).

Perpendicular endo-division relative to the cytoskeleton axis produced offspring cells with cell-shape changes and with proliferative freedom from loss of cell contact inhibition. These events occurred in pre-senescence, and for 3-D growths from old senescence escape-cells the cell-shape changes were augmented. This increase was suggested to arise from escape-cell retention to some degree of flat cell “amorphous-physiology” which does not express a polarity system (Walen 2011). Cancer-associated cell dedifferentiation to complete loss of cell polarity, expressed as “round cells”, currently has no definitive origin(s), implying high probability of endopolyploid cycling in tumorigenesis. This was recently shown from technologically advanced methodology (Puig et al. 2008) which is an important positive addition to the classical report on 4-chromatid chromosomal cycling in lymphomas (Levan and Hauschka 1953). Importantly, repeated endopolyploidizing events from presence of genomic damage (e.g., oncogene Myc activation) will increase cell-dedifferentiation from the innate process of endo-perpendicularity. For *Drosophila*-cells, perpendicular orientation in stem cell-division led to cancer-cell phenotypes of progenitor cells. For human cancers only one study of aggressive oral cancers hints at a similar relationship by descriptions of skewed divisions from “cytoskeleton defects” (Saunders et al. 2000). Genes for cytoskeleton

organization were found up-regulated (e.g., Rho-ATPases) at different stages of tumorigenic progression, but without associated mutational changes, supporting events of repeated endo-perpendicular divisions.

By-pass of dormancy periods is a general suggestion for recurrence of cancers following therapy, based on the *in vitro* immortalization-model (Vergel et al. 2011), but can equally well be suggested to arise from any of the described escape-cell mechanisms to mitotic events. A fair comparison to dormancy is TAS-treated-associated “senescence” with its development of 3-D tumor-like spheres from old escape-cells. This dramatic cellular change from primary, diploid cells inflicted by telomere dysfunction and genome-wide damage has no *in vitro* likeness (immortalization does not involve senescence escape). But, contrary to the 3-D growth’s origin from old senescence escape cells, increased tumorigenic potential of dormancy-escape cells is more recently thought to be effected from adjacent senescent, stromal secretory cells (Vergel et al. 2011). An additional route is the recent emphasis on autophagy with the dual ability to suppress or promote tumorigenesis (Young et al. 2009; Erenpreisa et al. 2011). Cells with a band of lysosomes around the nuclei (see above) may indicate that the “quality” of the senescent state might differ among the old senescent cells (Young et al. 2009).

The claim here that the senescent phase can lead to positive tumorigenesis from cells with endopolyploid division characteristics is firstly supported by endopolyploid chromosomal cycling of escape cells (Fig. 18.2a–g; Walen 2008), and secondly by the finding that endo-polyploid-reduction division was a source of tumor re-growth-cells (Puig et al. 2008). Moreover, chemo-agents against cancer cells were shown to induce endopolyploidization (giant cells) associated with induced senescence-like (TAS-like) morphology (Mosieniak and Sikora 2010). From these giant cells “late” genome reduced, escape cells propagated, showing mild aneuploid changes which is a recent disclosure associated with endopolyploid reductional division (Walen 2012). Fragmentation of giant cells (>8n) into genome reduced euploid

cells occurs in trophoblasts of the human placenta and also for normal diploid endopolyploid (>8n) cells in pre-senescence which showed mitotic capacity (Walen 2010). In agreement with Mosieniak and Sikora (2010) such genome progressions from endopolyploidy indicate a risk of the senescent state of promoting tumorigenesis, which is a potential challenge to recent suggestions of induced senescence as an anti-cancer therapy condition (Shay and Roninson 2004; Haugstetter et al. 2010; Collado and Serrano 2010; Vergel et al. 2011). There are warnings against prolonged induced dormancy periods unless, as observed, the immune system eliminates the senescent cells (Collado and Serrano 2010). The gap in our knowledge of the dormancy/remission period for reduction/curtailment of tumor recurrence has probability for rectification by molecular studies of the TAS-treated induced senescence. Such gained information can be applied to preserved biopsy materials for potential disclosures of markers relevant to targeted therapy (te Poele et al. 2002). But in the mean time “dormant tumor cells” – “represent a dangerous potential for tumor relapse” from escape cells (Collado and Serrano 2010).

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References

- Beausejour CM, Krotolica A, Galimi F, Narita M, Lowe SW, Yawen P, Campisi J (2003) Reversal of human senescence: roles of p16 and p53 pathways. *EMBO J* 22:4212–4222
- Bignold LP, Coghland BLD, Jersmann HPA (2007) David von Hansemann: contributions to oncology: context, comments, and translations. Birkhauser Verlag, Basel
- Ceol CJ, Pellman D, Zon LI (2007) APC and colon cancer: two hits for one. *Nat Med* 13:1286–1287
- Chen JH, Ozanne SE (2006) Deep senescent human fibroblasts show diminished DNA damage foci but retain checkpoint capacity to oxidative stress. *FEBS Lett* 580:6669–6673
- Collado M, Serrano M (2010) Senescence in tumors: evidence from mice and humans. *Nat Rev Cancer* 10:51–57
- Collado M, Gil J, Efeyan A, Guerra C, Schuhmacher AJ, Baradas M, Benguria A, Zaballos A, Flores JM,

- Barbacid M, Beach D, Serrano M (2005) Tumor biology: senescence in premalignant tumors. *Nature* 436:642
- Collado M, Blasco MA, Serrano M (2007) Cellular senescence in cancer and aging. *Cell* 130:223–233
- D'Amato F (1989) Polyploidy in cell differentiation. *Caryologia* 42:183–211
- Davoli T, Denchi EL, de Lange T (2010) Persistent telomere damage induces bypass of mitosis and tetraploidy. *Cell* 141:81–93
- Erenpreisa J, Salmira K, Huna A, Kosmacek EA, Cragg MS, Ianzini F, Anisimov AP (2011) Polyploid tumor cells elicit paraploid progeny through depolyploidizing divisions and regulated autophagy degradation. *Cell Biol Int* 35:687–695
- Freed JJ, Schatz SA (1969) Chromosome aberrations in cultured cells deprived of single essential amino acid. *Exp Cell Res* 55:393–409
- Haugstetter AM, Loddenkemper C, Lenze D, Grone J, Standfus C, Petersen I, Schmitt CA (2010) Cellular senescence predict treatment outcome in metastasized colorectal cancer. *Br J Cancer* 103:505–509
- Hayflick L, Moorhead PS (1961) The serial cultivation of human diploid cell strains. *Exp Cell Res* 25:585–621
- Levan A, Hauschka TS (1953) Endomitotic reduplication mechanisms in ascites tumors of the mouse. *J Natl Cancer Inst* 14:1–43
- Matsumura T, Zerrudo Z, Hayflick L (1979) Senescent human diploid cells in culture: survival, DNA synthesis and morphology. *J Gerontol* 34:328–334
- Mosieniak G, Sikora E (2010) Polyploidy: the link between senescence and cancer. *Curr Pharm Des* 16:734–740
- Puig P-E, Guilly M-N, Bouchot A, Droin N, Cathelin D, Bouyer F, Favier L, Ghiringhelli F, Kroemer G, Solary E, Martin F, Chauffert B (2008) Tumor cells can escape DNA-damaging cisplatin through DNA endoreduplication and reversible polyploidy. *Cell Biol Int* 32:1031–1043
- Roberson RS, Kussick SJ, Vallieres E, Chen S-YJ, Wu DY (2005) Escape from therapy-induced accelerated cellular senescence in p53-null lung cancer cells and in human lung cancers. *Cancer Res* 65:2795–2803
- Saunders WS, Shuster M, Huang X, Gharaibe B, Enyenihi AH, Petersen I, Gollin SM (2000) Chromosomal instability and cytoskeletal defects in oral cancer. *Proc Natl Acad Sci* 97:303–308
- Shay JW, Roninson IB (2004) Hallmarks of senescence in carcinogenesis and cancer therapy. *Oncogene* 23:2919–2933
- Smith PJ, Marquez N, Wiltshire M, Chappell S, Njoh K, Campbell L, Khan IA, Silvestre O, Errington RJ (2007) Mitotic bypass via an occult cell cycle phase following DNA topoisomerase II inhibition in p53 functional human tumor cells. *Cell Cycle* 6:2071–2081
- te Poele RH, Okorokov AL, Jardine L, Cummings J, Joel SP (2002) DNA damage is able to induce senescence in tumor cells in vitro and in vivo. *Cancer Res* 62:1876–1883
- Vergel M, Marin JJ, Estevez P, Carnero A (2011) Cellular senescence as a target in cancer control. *J Aging Res*. doi:10.4061/2011/725365
- Walen KH (1965) Spatial relationships in the replication of chromosomal DNA. *Genetics* 51:915–929
- Walen KH (2002) The origin of transformed cells: studies of spontaneous and induced cell transformation in cell cultures from marsupials, a snail, and human amniocytes. *Cancer Genet Cytogenet* 133:45–54
- Walen KH (2004) Spontaneous cell transformation: karyoplasts derived from multinucleated cells produce new cell growth in senescent human epithelial cell cultures. *In Vitro Cell Dev Biol Anim* 40:150–158
- Walen KH (2005) Budded karyoplasts from multinucleated fibroblast cells contain centrosome and change their morphology to mitotic cells. *Cell Biol Int* 29:1057–1065
- Walen KH (2006) Human diploid fibroblast cells in senescence: cycling through polyploidy to mitotic cells. *In Vitro Cell Dev Biol Anim* 42:216–224
- Walen KH (2007a) Origin of diplochromosomal polyploidy in near-senescent fibroblast cultures: heterochromatin, telomeres and chromosomal instability (CIN). *Cell Biol Int* 31:1447–1455
- Walen KH (2007b) Bipolar genome reductional division of human near-senescent, polyploidy fibroblast cells. *Cancer Genet Cytogenet* 173:43–50
- Walen KH (2008) Genetic stability of senescence reverted cells: genome reduction division of polyploid cells, aneuploidy and neoplasia. *Cell Cycle* 7:1623–1629
- Walen KH (2010) Mitosis is not the only distributor of mutated cells: non-mitotic enopolyploid cells produce reproductive genome reduced cells. *Cell Biol Int* 34:867–872
- Walen KH (2011) Normal human cell conversion to 3-D cancer-like growth: genome damage, endopolyploidy, senescence escape, and cell polarity change/loss. *J Cancer Ther* 2:181–189
- Walen KH (2012) Genome reversion process of endopolyploidy confers chromosome instability on descendent diploid cells. *Cell Biol Int* 36:1–9
- Young ARJ, Narita M, Ferreira M, Kirschner K, Sadaie M, Darot JFJ, Tavares S, Arakawa S, Shimizu S, Watt FM, Narita M (2009) Autophagy mediates the mitotic senescence transition. *Genes Dev* 23:798–803